Cell PRESS
Immunity
Previews

Coordinating Innate Immune Cells to Optimize Microbial Killing

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DOI 10.1016/j.immuni.2008.10.003

The mechanisms underlying innate immune cell trafficking and activation during infection remain incompletely defined. In this issue of *Immunity*, Kang et al. (2008) begin to reveal the cytokine and chemokine cascades that coordinate cellular responses induced by microbial pathogens.

Viruses, bacteria, protozoa, and fungi have evolved a range of mechanisms for invading mammalian hosts and causing infectious diseases. To cause infection and survive within a mammalian host, microbes activate virulence programs that enable them to survive or escape antimicrobial defenses. Most infectious diseases share a common theme: Microbial virulence mechanisms enable access to host compartments that are generally off limits to microbes and thereby induce innate immune and inflammatory responses that, in turn, promote the development and differentiation of adaptive immune responses. Kang et al. (2008) have extended our understanding of the innate immune response to infection with *Listeria monocytogenes* by providing a more exact spatial and temporal context for the contributions of CD11c dendritic cells, natural killer (NK) cells, inflammatory monocytes, and interleukin-12 (IL-12), IL-18, and interferon-γ (IFN-γ) in defense against intracellular bacterial infection (Kang et al., 2008).

L. monocytogenes, a Gram-positive bacterium that causes systemic infections in a broad range of mammals, has served as a useful model for dissecting the interactions between a microbial pathogen and the mammalian immune system (Pamer, 2004). In the case of *L. monocytogenes* infection, the microbe's virulence strategy begins with invasion of mammalian cells in order to escape extracellular antimicrobial factors. In contrast to most intracellular bacteria, however, *L. monocytogenes* goes one step further and escapes the vacuolar compartment by secreting listeriolysin O (LLO), a membranolytic protein that enables internalized bacteria to enter the host cell cytosol. Production of LLO and escape from the vacuole are essential for *L. monocytogenes* virulence, and bacteria lacking LLO are avirulent. In addition, *L. monocytogenes* lacking LLO do not induce in vivo inflammatory responses associated with virulent infection. In mice, priming of *L. monocytogenes*-specific $CD4^+$ and $CD8^+$ T cells, which provide long-term immunity to reinfection, is heavily influenced by innate inflammatory responses induced during the first few days of infection. Thus, cytosol invasion by *L. monocytogenes* induces a cascade of inflammatory events that include induction of cytokines and chemokines, organized recruitment of distinct cell populations, and early restriction of in vivo bacterial replication. These early events are essential for survival because, in their absence, bacterial growth occurs so rapidly that mice die within 72 to 96 hr, and they are essential for the effective priming and differentiation of responding T cells (Pamer, 2004).

Experiments with mice lacking different cytokines or cytokine and chemokine receptors have demonstrated that some of these molecules are essential for *L. monocytogenes* clearance whereas others impair early bacterial clearance. Mice lacking IL-10 or the receptor for type I interferons, for example, are more resistant to *L. monocytogenes* infection, indicating that these cytokines restrict protective immune defenses. In contrast, mice lacking IFN-γ, tumor necrosis factor (TNF), inducible nitric oxide synthase (iNOS), or the CCR2 chemokine receptor are far more susceptible to *L. monocytogenes* infection, indicating that these factors are essential for innate immune defense (Pamer, 2004). These findings led to a relatively simple model of innate immune defense against *L. monocytogenes*: bacteria infect macrophages and monocytes that, upon activation by IFN-γ and/or TNF, produce iNOS and kill intracellular bacteria.

Further studies, however, revealed that the actual mechanisms for in vivo defense against *L. monocytogenes* infection are more complex. Histologic analyses of infected spleens revealed that systemically administered *L. monocytogenes* are first localized to the marginal zone and are then carried by dendritic cells to the T cell zones of the splenic white pulp (Aoshi et al., 2008; Conlan, 1996). Concurrent with but independent of these early events in the spleen, CCR2-mediated signals promote emigration of monocytes from bone marrow into the blood stream and eventually to infected tissues, where monocytes differentiate to produce high amounts of TNF and iNOS and limit bacterial replication. This recruitment process results from the induction of the chemokines MCP-1 (monocyte chemoattractant protein 1) and MCP-3, but how infected tissues, such as the spleen and liver after *L. monocytogenes* infection, signal to the bone marrow to release $CCR2^+$ monocytes into the circulation remains unclear (Jia et al., 2008).

In this issue, Kang et al. have investigated the activation of newly recruited monocytes to the spleen and discovered some important and essential cytokine- and chemokine-mediated interactions between $CD11c^+$ DCs, NK cells, and $Ly6C^{hi}$ monocytes during the early innate immune response to *L. monocytogenes* infection. Staining of infected spleen for NK cells and inflammatory monocytes in combination with cell-depletion strategies

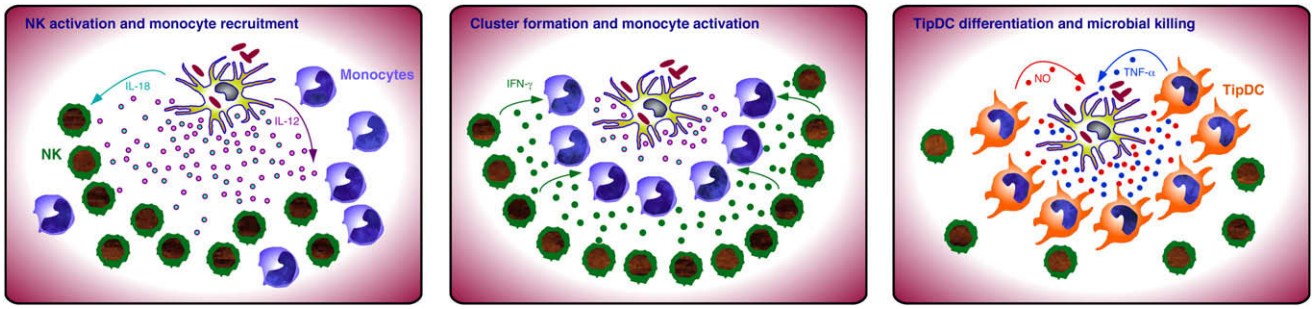

Figure 1. Sequential Activation of Innate Immune Responses during Bacterial Infection
After systemic administration of *L. monocytogenes*, dendritic cells (DCs) transport bacteria to the white-pulp areas of the spleen, where they initiate secretion of chemokines required for recruitment of natural killer (NK) cells and monocytes (left). DCs undergo MyD88-dependent activation and secrete IL-12 and IL-18 that in turn activate newly recruited NK cells to produce IFN-γ. Recruited cells are organized in clusters; monocytes are positioned in proximity to infected cells, and NK cells form a cuff at the periphery (middle). NK-derived IFN-γ induces monocyte activation. Monocyte activation leads to upregulation of MHC class II and iNOS expression and subsequent differentiation into TipDCs. TipDCs sense microbial infection in a MyD88-dependent manner and secrete TNF-α and nitric oxide (NO), ultimately leading to restriction of bacterial replication (right).

revealed that both cell populations independently colocalize at sites of infection and that NK cells form a cuff surrounding aggregated monocytes (Figure 1). Interestingly, recruitment of these two cell populations is concurrent, suggesting that, within the spleen, they might be responding to the same stimulus. The development of a new IFN-γ reporter mouse strain allowed the investigators to demonstrate that NK cells are the predominant producers of IFN-γ in the inflammatory aggregates and that IFN-γ induces differentiation of recruited monocytes into TNF- and iNOS-producing dendritic cells (Tip-DCs).

Kang et al. next investigated the role of CD11c-expressing dendritic cells in the early aggregation of NK cells and monocytes after *L. monocytogenes* infection. Remarkably, in mice lacking CD11c DCs, recruitment of monocytes and NK cells was almost completely turned off, suggesting that DCs orchestrate or at least facilitate the early inflammatory process. One caveat of this experiment, however, is that DCs play an important role in splenic infection, and as demonstrated in this study and a previous study (Neuenhahn et al., 2006), mice lacking DCs have markedly reduced splenic infection with *L. monocytogenes* after systemic inoculation. NK cell recruitment appears to be regulated, at least in part, by CCR5, a chemokine receptor that binds and responds to MIP-1α, MIP-1β, and RANTES. Thus, an attractive, straightforward, but unproven scenario is that DCs, in response to *L. monocytogenes* infection, secrete the chemokines that recruit NK cells and perhaps inflammatory monocytes to form the inflammatory aggregates.

Mice lacking MyD88 are highly susceptible to *L. monocytogenes* infection (Pamer, 2004). Toll-like receptor signaling is not required for monocyte recruitment to the infected spleen, but monocyte differentiation is impaired at the site of infection (Serbina et al., 2003). Kang et al. demonstrate that IFN-γ is diminished in the absence of MyD88-mediated signals. MyD88 signaling might be required for IL-12 and IL-18 secretion by $CD11c^+$ DCs and subsequent activation of NK cell-mediated IFN-γ production.

Recruitment of inflammatory cells and induction of chemokine secretion requires cytosol invasion by virulent *L. monocytogenes*. Avirulent *L. monocytogenes* that remain within the host cell vacuole, in contrast, induce far less chemokine secretion (Serbina et al., 2003). The identities of the receptor that detects cytosol invasion and the microbial ligands that stimulate chemokine secretion remain unknown. Kang et al. studied mice with deletions of some candidate cytosolic innate immune receptors or signaling molecules, Nod1, Nod2, ASC, and IFR3, but were not able to identify the pathway that initiates aggregation of NK cells and monocytes after *L. monocytogenes* infection. It is possible that these innate immune signaling pathways drive this process in parallel and that the absence of one pathway alone is insufficient to ablate the inflammatory response to cytosol invasion. This scenario will be important but difficult to address and might require complex breeding strategies for obtaining double, triple, and potentially quadruple gene-deleted mice.

Although recruitment and activation of monocytes has been most completely characterized in mice infected with *L. monocytogenes*, the mechanisms underlying this process are relevant to infections with other pathogens and monocyte-mediated inflammatory processes that cause human disease. Increasingly, monocyte recruitment has been shown to play an essential role in defense against a spectrum of different microbial infections, including Toxoplasmosis, Cryptococcosis, Salmonella, and Mycobacterium tuberculosis infection (Serbina et al., 2008). Monocyte recruitment and activation have also been implicated in vascular and demyelinating diseases such as atherosclerosis and multiple sclerosis (Gerard and Rollins, 2001), respectively, and TipDCs have been identified as important contributors to the pathogenesis of psoriasis (Lowes et al., 2005). Defining the mechanisms that promote monocyte recruitment and activation, therefore, might provide opportunities to promote defense against a range of infections and, in settings of overly robust inflammatory responses, opportunities to ameliorate pathologic states.

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